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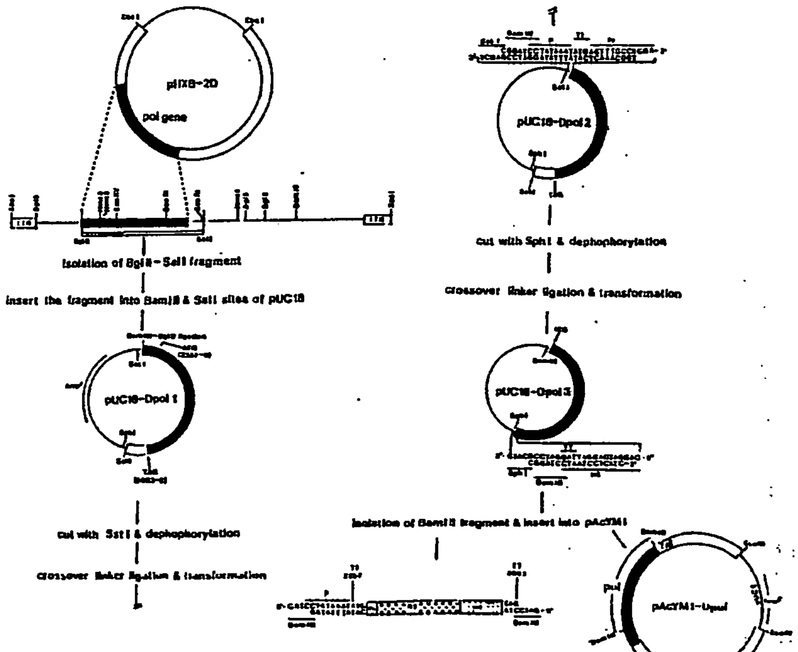
pIIXB-2D
pol gene
Isolation of BglII - SalI fragment
insert the fragment into BamHI & SalI sites of pUC18
pUC18-Dpol 1
cut with SstI & dephophorylation
crossover linker ligation & transformation
pUC18-Dpol 2
cut with SphI & dephophorylation
crossover linker ligation & transformation
pUC18-Dpol 3
Isolation of BamHI fragment & insert into pAcYM1
pol
pAcYM1-Dpol

(57) Abstract

A polypeptide having immunological activity for use as a diagnostic reagent and/or a vaccine component for the HIV virus. The polypeptide comprises a substantial portion of each of more than one of the constituent proteins coded for by the HIV-pol gene, namely HIV-pol protease, HIV-pol reverse transcriptase, HIV-pol RNase H and HIV-pol integrase.

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POLYPEPTIDE HAVING IMMUNOLOGICAL ACTIVITY FOR USE AS DIAGNOSTIC REAGENT AND/OR VACCINE

TECHNICAL FIELD

This invention relates to a polypeptide having immunological activity for use as a diagnostic reagent and/or a vaccine component.

BACKGROUND ART

Diagnostic kits for use in screening individuals for infection with human immunodeficiency virus (HIV) infection frequently include reagents comprising HIV antigens which are used to detect antibodies using known immunological techniques including ELISA, Western Blot, latex agglutination and immuno-luminescent and immuno-fluorescent techniques.

The effectiveness of such techniques however depends upon selection of suitable immunological reagents and one particular difficulty which arises is that particular reagents are often specific to individual strains or groups of strains of HIV. Thus, for example, known diagnostic reagents based upon HIV-1 may fail to detect antibodies resulting from an infection of a patient with HIV-2.

Similarly, in the production of vaccines designed to protect individuals against HIV infection, the use of antigens derived from one particular strain of HIV may fail to provide adequate protection against infection with other strains.

It is an object of the present invention to overcome such problems.

DISCLOSURE OF INVENTION

It has now been found that the product of expressing a substantial part of the HIV-pol gene in a suitable host has antigenic properties which allows the above-mentioned problems to be overcome.

Thus according to one aspect of the present invention there is provided the use as an antigenic reagent in the diagnostic test or as a vaccine component of a polypeptide comprising a substantial portion of each of more than one of the constituent proteins coded for by the HIV-pol gene.

Diagnostic kits and vaccines comprising said polypeptide form further aspects of the present invention.

The HIV-pol gene codes for four enzymes, namely a protease, a reverse transcriptase, a ribonuclease referred to as RNAse H and an enzyme referred to as Integrase.

It is believed that during infection of a T cell by HIV a full length precursor is expressed which is then cut up into the discrete proteins listed above. These have the following activities and (it is thought) act in the order indicated:-

Protease	Precursor Cleavage
Reverse Transcriptase	Preparation of viral DNA from viral RNA
RNAse H	Destruction of viral RNA leaving newly synthesised DNA
Integrase	Insertion of said DNA into host cell genome

According to a preferred aspect of the present invention, said constituent proteins are enzymes coded for by the HIV-pol gene and the polypeptide thus comprises a substantial portion of each of a plurality of enzymes selected from HIV-pol protease, HIV-pol reverse transcriptase, HIV-pol RNAse H and HIV-pol Integrase. Most preferably, the polypeptide comprises substantial portions of all four of said enzymes.

In vivo, the initial product of expressing the HIV-pol gene is cleaved into its individual elements by the protease. The active site for proteolytic activity occurs adjacent the NH_2-terminus of the expression product, corresponding to the 5'-end of the protease gene.

According to a preferred aspect of the present invention, the polypeptide omits at least that part of the amino acid sequence of the HIV-pol protease gene which codes for the active site responsible for proteolytic activity. By omitting this portion, the integrity of the polypeptide is maintained and it is less liable to degrade.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 is a schematic diagram showing the procedure of Example 1;

Figure 2 shows the results of electrophoresis tests carried out in the manner explained in Example 2; and

Figure 3 is a graph showing the results of the experiments carried out in Example 3.

BEST MODE FOR CARRYING OUT THE INVENTION

The HIV-pol gene of several strains of HIV-1 has been cloned and the corresponding amino acid sequences derived from the determined DNA sequences. The amino acid sequences of ten strains appear in the accompanying Table 1 at the end of this disclosure. In Table 1, the full sequence of strain HIV HXB2 is given, whereas for the other nine strains, only sequence differences are listed. As used herein, the term "constituent protein coded for by the HIV-pol gene" refers to a protein having sufficient amino acid homology with the sequence of HIV HXB2 appearing in the accompanying Table so as to result in antibodies raised against the protein cross-reacting with a polypeptide consisting of the precise amino acid sequence of HIV HXB2.

The HIV-pol gene can be expressed to produce the desired polypeptide by various techniques, e.g. some or all of the baculovirus techniques described in U.S. Patent 4,745,051 to Gale E. Smith et al issued on May 17, 1988; Baculovirus Vectors for Expression of Foreign Genes by C. Yong Kang, Advances in Virus Research, Vol. 35, pp 177-192, Academic Press Inc., 1988; A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures, Max D. Summers and Gale E. Smith, May 1987, Texas A&M University; and Baculoviruses as Gene Expression Vectors, Lois K. Miller, Ann. Rev. Microbiol. 42, pp 177-1991; the disclosures of which are incorporated herein by reference. However our Canadian Patent Application Serial No. 591,908 filed on 23rd February 1989 (and equivalent British Patent Application Serial No. 89 04426.7 filed on February 27, 1989 and US Patent Application Serial No. 316,768 filed on February 28, 1989) describes and claims an improved baculovirus expression system capable of producing foreign gene proteins at high levels and the use of this expression system is particularly preferred for expressing the polypeptide of the present invention.

The process disclosed in our Canadian patent employs a recombinant baculovirus containing at least a major part of a polyhedrin gene promoter region, a transcription termination sequence of a polyhedrin structural gene, a foreign structural gene (e.g. an HIV-pol gene) having a translation start codon followed by coding sequences and a translation stop codon. The foreign gene is located between the promoter region and the termination sequence. Immediately upstream of the start codon there is a putative insect cell ribosome binding site for the polyhedrin gene effective for overcoming resistance of susceptible insect cells to express the foreign gene at a high level. The putative ribosome binding site comprises at least the final four nucleotides of the sequence 5'-ACCTATAAAT-3'.

Example 3 of the Canadian application describes the production of the pol protein of HIV-1 in a baculovirus expression system based on Autographa californica nucleopolyhedrosis virus (ACNPV) and specifies that a recombinant baculovirus designated ACNPV-HIV-YK-pol has been deposited at the American Type Culture Collection of 12301 Parklawn Drive, Rockville MD 20852, USA under Accession No. ATCC VR 2233. Deposit was made on November 30, 1988. The disclosure of our Canadian Patent Application Serial No. 591,908 is incorporated herein by reference.

Utilising the procedures described in Example 3 of Canadian Patent Application Serial No.591,908, a polypeptide comprising the protease, RNAse H and Integrase enzymes of HIV strain HIV-XB2 may be produced.

The polypeptide can be used as a diagnostic reagent or vaccine component in ways known to persons skilled in the art, e.g. by the techniques indicated in the publication entitled Clinica, Testing for HIV and AIDS, The Next Five Years, George Street Publications Ltd., Richmond, Surrey, UK, the disclosure of which is incorporated herein by reference.

The invention is illustrated in more detail by the following Examples. Example 1 illustrates the production of a modified recombinant plasmid pUC18-Dpol3 having a 273 bp deletion at the 5'-terminus and its expression as polypeptide lacking the first 91 amino acids at the NH_2-terminus of the HIV-pol protease. Examples 2 and 3 relate to the expression of the polypeptide and its use as a diagnostic reagent.

EXAMPLE 1

Construction of baculovirus transfer vector containing HIV-1 pol gene with 273 bp deletion at 5' terminus

As illustrated in Figure 1, the BglII and SalI fragment of plasmid pHXB-2D containing the HIV-1 pol coding region was isolated and inserted into BamHI and SalI sites of pUC18. The resulting recombinant plasmid (pUC18-Dpol 1) was cut with Sst1 and dephosphorylated. A synthetic double-stranded crossover linker containing a Sst1 cohesive end, a BamHI site, the putative insect _Spodoptera frugiperda_ (SF9) cell ribosome binding site (P) and 15 nucleotides of the homology searching sequences which overlaps with the 5' terminus of the pol gene was ligated at the Sst1 site and transformed. The recombinant plasmid, (pUC18-Dpol 2) was isolated, digested with sPH1, dephosphorylated and ligated with another crossover linker DNA containing SphI cohesive end at the 3' terminus, BamH1 site and 15 nucleotides of the homology searching sequences which recognise the 3' terminus of the pol gene. The resulting recombinant plasmid (pUC18-Dpol 3) contains the putative SF9 cell ribosome binding site (P) followed with pol open reading frame starting with the first ATG (TI) codon (map unit 2357-2359) in the pol gene and the translation termination (TT) codon TAG (map unit 5093-5095). This whole cassette was flanked with BamH1 sites. The BamH1 fragment was isolated and inserted into the BamH1 site of the pAcYM1 baculovirus transfer vector (pAcYM1-Dpol). The pAcYM1-Dpol transfer vector DNA was used to co-transfect SF9 cells with wild type AcNPV DNA to isolated recombinant AcNPV HIV-YK pol virus.

EXAMPLE 2

Expression of pol gene products by recombinant baculoviruses

Recombinant AcNPV-HIVWHpol contains an insert comprising essentially the whole DNA sequence of the HIV-pol gene (see Table 2 at the end of the present disclosure). When expressed, the resulting full length gene product of the HIV-pol gene is "processed", i.e. the proteolytic active site of the HIV pol protease gene cleaves the protein into 66 kD, 51 kD and 32 kD fragments.

By way of comparison, recombinant AcNPV-HIVYKpol (see Table 3 at the end of the present disclosure) omits NH_2-terminal amino acid sequences containing the proteolytic active site of the HIV-pol protease. When expressed, the resulting gene product is not "processed", i.e. the ~ 95 kD protein remains intact.

The following experiments illustrate this.

Uninfected S. frugiperda (SF9) cells, or SF9 cell infected with recombinant baculoviruses AcNPV-HIVWHpol, AcNPV-HIVYKpol or with wild-type AcNPV, were harvested after 72 hours of infection. Lysates of the infected or uninfected cells were electrophoresed in a 12% polyacrylamide Laemmli gel and proteins are identified by either Coomassie blue staining (S) or Western blot analyses (W) using the standard HIH HIV positive immunoglobulin. As shown in Figure 2, lanes 1, 2 and 3 represents the lysates of AcNPV-HIVYHpol recombinant virus infected cells, lanes 4, 5 and 6 represent the lysates of AcNPV-HIVYKpol recombinant virus infected cells, lane 7 shows the wild-type AcNPV infected cell lysate, lane 8 shows uninfected cell lysate and lane 9 shows molecular weight markers. Lane 3 and 6 show the whole cell lysate, lanes 2 and 5 show proteins in the infected cell nuclei and lanes 1 and 4 show proteins in the infected cell cytoplasm. P denotes polyhedrin protein and arrows show 95K Dal uncleaved pol gene product representing 91 amino acid deletion of protease produced by AcNPV-HIVYKpol virus and 66K Dal, 51 K Dal and 33K Dal processed pol gene products in AcNPV-HIVYHpol virus infected cells.

EXAMPLE 3

A. Production of pol gene product

Recombinant ACNPV-HIVYKpol virus infected Spodoptera frugiperda (SF9) cells were harvested 4 days after infection. Nuclei of infected cells containing most of the pol gene product were isolated by treating the infected cells with 0.1% Triton X-100 and 0.5% NP40 on ice for 20 minutes followed by centrifugation at 750 g for 10 minutes. The pelleted nuclei were denatured with 1% SDS in TRIS-HCl pH 8.0 at room temperature for 30 minutes. The cellular DNAs were removed by ethanol precipitation using 2 volumes of 100% ethanol. The SDS in the solution were removed by addition of 25 mM KCL incubated at 4°C for 30 minutes followed by centrifugation at 12,700 g for 15 minutes. The pol gene product in the supernatant was used for anti-pol ELISA.

B. Detection of HIV antibodies by ELISA

The pol antigen was diluted in PBS and dispensed in a microtiter plate (Nunc cat 269620). The concentration of pol to coat plates was determined empirically on the strength of bands on polyacrylamide gels.

The concentration of pol necessary to coat one well was between 1 and 10 µg.

The plate was covered and incubated at 4°C. The time of incubation varied between 12 and 24 hrs without no apparent differences in reactivity.

The plates were then washed three times in PBS tween 20 employing a Skatron plate washer.

Various standards, NIH HIV+ immunoglobulin (NIH STD), pool HIV+ plasma (PAT STD) and plasma from non-infected individuals (NS) were employed. The standards were diluted beginning at 1:200 for NIH STD, and 1:10 for PAT STD and NS. Unknowns were tested usually at 1:50 but dilutions as high as 1:10 can be employed.

All samples were inactivated before testing. Normal sera were processed in the same fashion as sera from AIDS patients. The inactivation was performed with 4'-aminoethyltrioxsalen- hydrochloride (AMT) from Lee Biomolecular Research Inc. (San Diego, California cat 231) and an ultra violet light trans-illuminator (Spectroline model TC-365, Fisher Scientific Ottawa Ont.). The AMT was reconstituted in 50% ethanol at 1 μg/ml. The sera was aliquoted in Eppendorf tubes and for every 100 μl of serum or plasma, 10 μl of AMT was added to the sample. The samples were layed in the transilluminator and irradiated for 5 minutes. An additional 10 μl of AMT was added to the sample and the samples were irradiated for a further 5 minutes. The samples were inactivated by this procedure.

The incubation time of the human-anti-pol was 30 to 40 minutes at room temperature (23°C) (the time of incubation found to be quite critical). Therefore, all dilutions of standards (negative and positive) and unknowns was performed in a separate plate. Once all dilutions were done, the dilutions (100 μl) were transferred to the ELISA plate coated with pol employing a multichannel pipettor. All dilutions were with PBS Tween 20 (0.1%).

The state of the serum or plasma sample was found to be important. Samples repeatedly frozen and thawed usually gave higher backgrounds. This was especially evident with samples from normal individuals.

The plates were washed three times in PBS-Tween 20 after the 30 minute incubation with the first antibody.
A Skatron II plate washer was employed for this purpose.

The second antibody used (goat anti-human Ig linked to horse radish peroxidase) was an affinity purified reagent obtained from Tago Diagnostics (Inter Medico To DNT cat 2393). An appropriate dilution was determined experimentally (approximately 1:2,000) is made in PBS-Tween 20 (0.1%). 100 µl was dispensed into the wells except for one which will be employed as a blank for the plate reader. The plate was incubated for 1 hour at room temperature.

The plates were washed three times with PBS-Tween 20 employing the Skatron II plate washer.

Freshly prepared substrate (100 µl) was added to the wells and after 20 minutes the reaction stopped with the addition of 100 µl of 0.07M H2SD4.

The plate was read at 450 nm in the BIOTEK BL/310 ELISA plate reader. A hard copy of the data was obtained from the reader and the data also stored directly onto computer diskette for further processing by the Anelisar program.

Additionally, controls were also performed on each plate. In two or three wells no serum or plasma was added. In one well no primary or secondary antibodies were added but substrate was. This well was employed to blank the ELISA plate reader. The remaining wells were employed to determine the extent of binding of the secondary antibody (Goat anti-HIg-HRPO) to POL. Thus, these wells received no primary antibody but secondary antibody and substrate with the appropriate washes in between each incubation. Usually the value of this latter control is below 0.1000 OD.

The results are shown in Figure 3.

The following materials were use for the anti-pol ELISA procedure

Buffers

Phosphate Buffered Saline (PBS)

Na_2HPO_4 (dibasic anhydrous)	13.6 g
NaH_2PO_4 (monobasic)	2.4 g
NaCl	90.0 g

Salts are dissolved in 8 litres of distilled deionized water and pH is adjusted to 7.2 with NaOH or HCl. This buffer is employed as coating buffer, diluent and washing buffer. The latter two buffers are modified as indicated below.

Diluent for primary and secondary antibodies and washing buffer

PBS + 0.1% Tween 20 (Sigma, St. Louis MO) (0.1 ml Tween 20 + 100 ml PBS). The diluent buffer is made up daily.

Substrate buffer

Equal volumes of 0.1M Na_2HPO_4 (0.709 g/50 ml) and 0.1M citric acid (0.960 g/50 ml). The pH is adjusted to 4.0 with NaOH or HCl. The substrate buffer is made up weekly.

Substrate

A tablet (2 mg) of o-phenylenediamine (Sigma cat. P6787) is dissolved into 10 ml of substrate buffer. Hydrogen peroxide (4 μl of 30%) is added to the solution just prior to plating. The solution should be kept in the dark as much as possible.

Stopping reagent

The enzymatic reaction is stopped with 0.07M H_2SO_4.

It is a particularly advantageous feature of the polypeptides, the use of which is described herein, that they cross-react with antibodies against diverse strains of HIV. Thus, for example, the polypeptides described herein based on HIV-1 can cross-react with antibodies raised against various strains of HIV-1 and HIV-2. Thus they may be used in diagnostic kits for detecting either virus category. Similarly, in vaccines they can provide broad-spectrum protection.

Industrial Applicability

As will be apparent from the above, the present invention can be used in the medical field for testing for HIV infection and for immunizing against HIV infection, as well as for other diagnostic or prognostic purposes.

TABLE 1

HIV-1 pol protein sequence of HIVHXB2 virus
Data from Human Retroviruses and AIDS 1988
Los Alamos National Laboratory

AcNPV-HIVWHpol

```
HIVHXB2Met PhePheArgGluAspLeuAlaPheLeuGlnGlyLysAlaArgGluPheSerSerGlu...   19
 HIVBH102  ---------------------------------------------------------Gln   20
 HIVBH5    ---------------------------------------------------------Gln   20
 HIVPV22   ---------------------------------------------------------Gln   20
 HIVBRU    ---------------------------------------------------------Gln   20
 HIVMN     .........................................................---    0
 HIVSF2    ------------------------------------------------------------   19
 HIVRF     ------------Asn---------Pro------------------Leu------------   19
 HIVMAL    ------------Asn---------Pro---------------------Pro---------   19
 HIVELI    ------------Asn---------Pro------------Gly---Leu---ProLys---   19

 HIVHXB2   .................................GlnThrArgAlaAsnSerProThrArg   28
 HIVBH102  ThrArgAlaAsnSerProThrIleSerSerGlu---------------------------   40
 HIVBH5    ThrArgAlaAsnSerProThrIleSerSerGlu---------------------------   40
 HIVPV22   ThrArgAlaAsnSerProThrIleSerSerGlu---------------------------   40
 HIVBRU    ThrArgAlaAsnSerProThrIleSerSerGlu---------------------------   40
 HIVMN     ---------------------------------...........................    0
 HIVSF2    ------------------------------------------------------------   28
 HIVRF     ------------------------------------------------------------   28
 HIVMAL    ---------------------------------------------------------Ser   28
 HIVELI    ---------------------------------------------------------Ser   28

 HIVHXB2   ArgGluLeuGlnValTrpGlyArgAspAsnAsnSerProSerGluAlaGlyAlaAspArg   48
 HIVBH102  ------------------------------------------------------------   60
 HIVBH5    ------------------------------------------------------------   60
 HIVPV22   ------------------------------------------------------------   60
 HIVBRU    ------------------------------------Leu---------------------   60
 HIVMN     ............................................................    0
 HIVSF2    ---------------------GlyGlu---------Leu---------------------   48
 HIVRF     ------------------------------...---Leu---------------Glu---   47
 HIVMAL    ---------Arg------------Gly---...LysThrLeu------Thr------Glu---   47
 HIVELI    ---------Arg------------------...---ProLeu---LysThr------Glu---   47

 HIVHXB2   GlnGlyThrValSerPheAsnPheProGlnValThrLeuTrpGlnArgProLeuValThr   68
 HIVBH102  ------------------------------Ile---------------------------   80
 HIVBH5    ------------------------------Ile---------------------------   80
 HIVPV22   ------------------------------Ile---------------------------   80
 HIVBRU    ------------------------------Ile---------------------------   80
 HIVMN     ............................................................    0
 HIVSF2    ------------------------------Ile---------------------------   68
 HIVRF     ------------------Ser---------Ile------------------Ile------   67
 HIVMAL    ------Ile---------Ser---------Ile------------------Val------   67
 HIVELI    ------------------------------Ile------------------------Ala   67
```

SUBSTITUTE SHEET

Table 1 cont'd

```
            <- gag cds end
HIVHXB2   IleLysIleGlyGlyGlnLeuLysGluAlaLeuLeuAspThrGlyAlaAspAspThrVal   88
HIVBH102  ------------------------------------------------------------  100
HIVBH5    ------------------------------------------------------------  100
HIVPV22   ------------------------------------------------------------  100
HIVBRU    ------------------------------------------------------------  100
HIVMN     ............................................................    0
HIVSF2    ---Arg------------------------------------------------------   88
HIVRF     Val---------------------------------------------------------   87
HIVMAL    ValArgVal---------------------------------------------------   87
HIVELI    ------------------------------------------------------------   87

             AcNPV-HIVYKpol starts
HIVHXB2   LeuGluGluMetSerLeuProGlyArgTrpLysProLysMetIleGlyGlyIleGlyGly  108
HIVBH102  ------------------------------------------------------------  120
HIVBH5    ------------------------------------------------------------  120
HIVPV22   ------------------------------------------------------------  120
HIVBRU    ------------------------------------------------------------  120
HIVMN     .........---Asn------Arg------------------------------------   17
HIVSF2    ------------Asn---------Lys---------------------------------  108
HIVRF     ------------Asn---------Lys---------------------------------  107
HIVMAL    ---------IleAsn---------Lys---------------------------------  107
HIVELI    ------------Asn---------Lys---------------------------------  107

HIVHXB2   PheIleLysValArgGlnTyrAspGlnIleLeuIleGluIleCysGlyHisLysAlaIle  128
HIVBH102  ------------------------------------------------------------  140
HIVBH5    ------------------------------------------------------------  140
HIVPV22   ------------------------------------------------------------  140
HIVBRU    ------------------------------------------------------------  140
HIVMN     ------------------------------Thr---Gly---------------------   37
HIVSF2    ------------------------------ProVal------------------------  128
HIVRF     ------------------------------------------------------------  127
HIVMAL    ------------------------------------------------Lys---------  127
HIVELI    ------------------------------Pro---------------Gln---------  127

HIVHXB2   GlyThrValLeuValGlyProThrProValAsnIleIleGlyArgAsnLeuLeuThrGln  148
HIVBH102  ------------------------------------------------------------  160
HIVBH5    ------------------------------------------------------------  160
HIVPV22   ------------------------------------------------------------  160
HIVBRU    ------------------------------------------------------------  160
HIVMN     ------------------------------------------------------------   57
HIVSF2    ------------------------------------------------------------  148
HIVRF     ------------------------------------------------------------  147
HIVMAL    ------Ile---------------------------------------Met---------  147
HIVELI    ------------------------------------------------------------  147
```

Table 1 cont'd

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HIVHXB2	IleGlyCysThrLeuAsnPheProIleSerProIleGluThrValProValLysLeuLys	168
HIVBH102	--	180
HIVBH5	--	180
HIVPV22	--	180
HIVBRU	--	180
HIVMN	Leu---	77
HIVSF2	--	168
HIVRF	--	167
HIVMAL	--	167
HIVELI	--	167

HIVHXB2	ProGlyMetAspGlyProLysValLysGlnTrpProLeuThrGluGluLysIleLysAla	188
HIVBH102	--	200
HIVBH5	--	200
HIVPV22	--	200
HIVBRU	--	200
HIVMN	--	97
HIVSF2	--	188
HIVRF	--	187
HIVMAL	------------------Arg---------------------------------------	187
HIVELI	--	187

HIVHXB2	LeuValGluIleCysThrGluMetGluLysGluGlyLysIleSerLysIleGlyProGlu	208
HIVBH102	--	220
HIVBH5	--	220
HIVPV22	--	220
HIVBRU	--	220
HIVMN	---Ile--	117
HIVSF2	--	208
HIVRF	--	207
HIVMAL	---Thr---------LysAsp---------------------Leu---------------	207
HIVELI	---Thr------------Asp------------------------Arg------------	207

HIVHXB2	AsnProTyrAsnThrProValPheAlaIleLysLysLysAspSerThrLysTrpArgLys	228
HIVBH102	--	240
HIVBH5	--	240
HIVPV22	--	240
HIVBRU	--	240
HIVMN	--	137
HIVSF2	--	228
HIVRF	--	227
HIVMAL	--	227
HIVELI	------------------Ile---------------------------------------	227

Table 1 cont'd

HIVHXB2	LeuValAspPheArgGluLeuAsnLysArgThrGlnAspPheTrpGluValGlnLeuGly	248
HIVBH102	--	260
HIVBH5	------------------------Arg---------------------------------	260
HIVPV22	--	260
HIVBRU	--	260
HIVMN	---------------------------Lys------------------------------	157
HIVSF2	--	248
HIVRF	--	247
HIVMAL	------Asn---	247
HIVELI	--	247

HIVHXB2	IleProHisProAlaGlyLeuLysLysLysLysSerValThrValLeuAspValGlyAsp	268
HIVBH102	--	280
HIVBH5	--	280
HIVPV22	--	280
HIVBRU	--	280
HIVMN	--	177
HIVSF2	--	268
HIVRF	--	267
HIVMAL	--	267
HIVELI	--	267

HIVHXB2	AlaTyrPheSerValProLeuAspGluAspPheArgLysTyrThrAlaPheThrIlePro	288
HIVBH102	--	300
HIVBH5	--	300
HIVPV22	--	300
HIVBRU	--	300
HIVMN	------------------------Lys---------------------------------	197
HIVSF2	------------------------Lys---------------------------------	288
HIVRF	------------------------LysGlu------------------------------	287
HIVMAL	--	287
HIVELI	---Ser	287

HIVHXB2	SerIleAsnAsnGluThrProGlyIleArgTyrGlnTyrAsnValLeuProGlnGlyTrp	308
HIVBH102	--	320
HIVBH5	------------------------SerGly------------------------------	320
HIVPV22	--	320
HIVBRU	--	320
HIVMN	--	217
HIVSF2	--	308
HIVRF	---------------------Arg------------------------------------	307
HIVMAL	--	307
HIVELI	--	307

Table 1 cont'd

HIVHXB2	LysGlySerProAlaIlePheGlnSerSerMetThrLysIleLeuGluProPheArgLys	328
HIVBH102	--Lys---	340
HIVBH5	--	340
HIVPV22	--	340
HIVBRU	--	340
HIVMN	--	237
HIVSF2	--	328
HIVRF	--Lys---	327
HIVMAL	---Thr	327
HIVELI	--	327

HIVHXB2	GlnAsnProAspIleValIleTyrGlnTyrMetAspAspLeuTyrValGlySerAspLeu	348
HIVBH102	--	360
HIVBH5	--	360
HIVPV22	--	360
HIVBRU	--	360
HIVMN	--	257
HIVSF2	--	348
HIVRF	---------Glu--	347
HIVMAL	Lys------Glu--	347
HIVELI	---------GluMet---	347

HIVHXB2	GluIleGlyGlnHisArgThrLysIleGluGluLeuArgGlnHisLeuLeuArgTrpGly	368
HIVBH102	--	380
HIVBH5	--	380
HIVPV22	--	380
HIVBRU	--	380
HIVMN	------------------Ala------------------Arg------------------	277
HIVSF2	--	368
HIVRF	------------------Ile------------------Glu---------Lys------	367
HIVMAL	---------------------------------------Glu---------Lys------	367
HIVELI	------------------------------Lys------Glu------------------	367

HIVHXB2	LeuThrThrProAspLysLysHisGlnLysGluProProPheLeuTrpMetGlyTyrGlu	388
HIVBH102	--	400
HIVBH5	Phe---	400
HIVPV22	--	400
HIVBRU	--	400
HIVMN	Phe---	297
HIVSF2	Phe---	388
HIVRF	Phe---	387
HIVMAL	Phe---	387
HIVELI	Phe---Arg---	387

Table 1 cont'd

HIVHXB2	LeuHisProAspLysTrpThrValGlnProIleValLeuProGluLysAspSerTrpThr	408
HIVBH102	--	420
HIVBH5	---------------------Ile------------------------------------	420
HIVPV22	--	420
HIVBRU	--	420
HIVMN	--	317
HIVSF2	---------------------------------Met------------------------	408
HIVRF	--	407
HIVMAL	---------------------------------Gln------Asp---Glu---------	407
HIVELI	---------------------------Ser---Lys------------Glu---------	407

HIVHXB2	ValAsnAspIleGlnLysLeuValGlyLysLeuAsnTrpAlaSerGlnIleTyrProGly	428
HIVBH102	--	440
HIVBH5	--	440
HIVPV22	--	440
HIVBRU	--	440
HIVMN	--Ala---	337
HIVSF2	--Ala---	428
HIVRF	--Ala---	427
HIVMAL	--	427
HIVELI	---------------Asn------GluArg------------------------------	427

HIVHXB2	IleLysValArgGlnLeuCysLysLeuLeuArgGlyThrLysAlaLeuThrGluValIle	448
HIVBH102	--	460
HIVBH5	--	460
HIVPV22	--	460
HIVBRU	--	460
HIVMN	---------Lys--	357
HIVSF2	---------Lys--	448
HIVRF	---------Lys---Val	447
HIVMAL	---------Lys------------------------Ala------------AspIleVal	447
HIVELI	--	447

HIVHXB2	ProLeuThrGluGluAlaGluLeuGluLeuAlaGluAsnArgGluIleLeuLysGluPro	468
HIVBH102	--	480
HIVBH5	--	480
HIVPV22	--	480
HIVBRU	--	480
HIVMN	--	377
HIVSF2	--	468
HIVRF	Gln------Lys--	467
HIVMAL	---------Ala--	467
HIVELI	--	467

Table 1 cont'd

HIVHXB2	ValHisGlyValTyrTyrAspProSerLysAspLeuIleAlaGluIleGlnLysGlnGly	488
HIVBH102	--	500
HIVBH5	--	500
HIVPV22	--	500
HIVBRU	--	500
HIVMN	---Val------------	397
HIVSF2	------Glu---------------------------Val---------------------	488
HIVRF	--	487
HIVMAL	--	487
HIVELI	--	487
HIVHXB2	GlnGlyGlnTrpThrTyrGlnIleTyrGlnGluProPheLysAsnLeuLysThrGlyLys	508
HIVBH102	--	520
HIVBH5	--	520
HIVPV22	--	520
HIVBRU	--	520
HIVMN	--	520
HIVSF2	--	417
HIVRF	--	508
HIVMAL	---------------------------------GlnTyr---------------------	507
HIVELI	His---	507
		507
HIVHXB2	TyrAlaArgMetArgGlyAlaHisThrAsnAspValLysGlnLeuThrGluAlaValGln	528
HIVBH102	--	540
HIVBH5	--	540
HIVPV22	--	540
HIVBRU	---------Thr--	540
HIVMN	--	540
HIVSF2	--	437
HIVRF	--	528
HIVMAL	---------IleLysSer--	527
HIVELI	---Ala------------	527
		527
HIVHXB2	LysIleThrThrGluSerIleValIleTrpGlyLysThrProLysPheLysLeuProIle	548
HIVBH102	--	560
HIVBH5	--	560
HIVPV22	--	560
HIVBRU	--	560
HIVMN	------Ala---------------------------------------Arg---------	457
HIVSF2	---ValSer---------------------------Ile---------------------	548
HIVRF	---ValAla---	547
HIVMAL	------AlaGln------------------------------------Arg---------	547
HIVELI	Arg---Ser------------------------Arg------------Arg---------	547

Table 1 cont'd

```
HIVHXB2   GlnLysGluThrTrpGluThrTrpTrpThrGluTyrTrpGlnAlaThrTrpIleProGlu   568
HIVBH102  ------------------------------------------------------------   580
HIVBH5    ------------------------------------------------------------   580
HIVPV22   ------------------------------------------------------------   580
HIVBRU    ------------------------------------------------------------   580
HIVMN     ------------------------------------Thr+++------------------   477
HIVSF2    ------------------Ala------Met------------------------------   568
HIVRF     ------------------Ala---------------------------------------   567
HIVMAL    ------------------Ala---------------------------------------   567
HIVELI    ---------------------------Ala------------------------------   567

HIVHXB2   TrpGluPheValAsnThrProProLeuValLysLeuTrpTyrGlnLeuGluLysGluPro   588
HIVBH102  ------------------------------------------------------------   600
HIVBH5    ------------------------------------------------------------   600
HIVPV22   ------------------------------------------------------------   600
HIVBRU    ------------------------------------------------------------   600
HIVMN     ------Val---------------------------------------------------   497
HIVSF2    ------------------------------------------------------------   588
HIVRF     ------------------------------------------------------------   587
HIVMAL    ---------------------------------------------------Thr------   587
HIVELI    ------------------------------------------------------------   587

HIVHXB2   IleValGlyAlaGluThrPheTyrValAspGlyAlaAlaAsnArgGluThrLysLeuGly   608
HIVBH102  ------------------------------------------------------------   620
HIVBH5    ---------------------------------------Ser------------------   620
HIVPV22   ---------------------------------------------------Arg------   620
HIVBRU    ---------------------------------------Ser------------------   620
HIVMN     ------------------------------------------------------Lys---   517
HIVSF2    ------------------------------------------------------------   608
HIVRF     ---Ile------------------------------------------------------   607
HIVMAL    ------------------------------------------------------Lys---   607
HIVELI    ---Ile------------------------------------------------------   607

HIVHXB2   LysAlaGlyTyrValThrAsnArgGlyArgGlnLysValValThrLeuThrAspThrThr   628
HIVBH102  ---------------------Lys------------------Pro------Asn------   640
HIVBH5    ---------------------------------------------------His------   640
HIVPV22   ------------Leu------Lys------------------Pro------Asn------   640
HIVBRU    ------------------------------------------------------------   640
HIVMN     ------------------------------------------Ser---------------   537
HIVSF2    ------------------Asp---------------------SerIleAla---------   628
HIVRF     ------------------Asp---------------------Ser---------------   627
HIVMAL    ------------------Asp---------------------Ser------Glu------   627
HIVELI    ------------------Asp---------------------Pro---------------   627
```

Table 1 cont'd

HIVHXB2	AsnGlnLysThrGluLeuGlnAlaIleTyrLeuAlaLeuGlnAspSerGlyLeuGluVal	648
HIVBH102	--	660
HIVBH5	---------------------------His------------------------------	660
HIVPV22	--	660
HIVBRU	---------------------------His------------------------------	660
HIVMN	---------------------------His------------------------------	660
HIVSF2	---------------------------His------------------------------	557
HIVRF	---------------------------His------------------------------	648
HIVMAL	---------------------------His---------------Ser------------	647
HIVELI	---------------------------Asn------------------------------	647
HIVHXB2	AsnIleValThrAspSerGlnTyrAlaLeuGlyIleIleGlnAlaGlnProAspGlnSer	668
HIVBH102	--Lys---	680
HIVBH5	--Lys---	680
HIVPV22	--	680
HIVBRU	--Lys---	680
HIVMN	--Lys---	680
HIVSF2	--Lys---	577
HIVRF	--Lys---	668
HIVMAL	--Lys---	667
HIVELI	--Lys---	667
HIVHXB2	GluSerGluLeuValAsnGlnIleIleGluGlnLeuIleLysLysGluLysValTyrLeu	688
HIVBH102	--	700
HIVBH5	--	700
HIVPV22	---Gln------------	700
HIVBRU	--	700
HIVMN	---------------Ser--	597
HIVSF2	---------------Ser--	688
HIVRF	---------------Ser--	687
HIVMAL	---------Ile---------------------------Gln---Asp------------	687
HIVELI	--	687
HIVHXB2	AlaTrpValProAlaHisLysGlyIleGlyGlyAsnGluGlnValAspLysLeuValSer	708
HIVBH102	--	720
HIVBH5	--	720
HIVPV22	--	720
HIVBRU	--	720
HIVMN	--	617
HIVSF2	--	708
HIVRF	--Arg---------	707
HIVMAL	Ser---	707
HIVELI	--	707

Table 1 cont'd

HIVHXB2	AlaGlyIleArgLysValLeuPheLeuAspGlyIleAspLysAlaGlnAspGluHisGlu	728
HIVBH102	---------------Ile--	740
HIVBH5	---------------Ile------------------------------Glu---------	740
HIVPV22	---------------Ile--	740
HIVBRU	--	740
HIVMN	--GluAsp------	637
HIVSF2	---------------------------Asn------------------Glu---------	728
HIVRF	Thr---	727
HIVMAL	Ser---Glu---------	727
HIVELI	Gln---Glu---------	727

HIVHXB2	LysTyrHisSerAsnTrpArgAlaMetAlaSerAspPheAsnLeuProProValValAla	748
HIVBH102	--	760
HIVBH5	--	760
HIVPV22	--	760
HIVBRU	--	760
HIVMN	---Ile------	657
HIVSF2	--	748
HIVRF	--	747
HIVMAL	---Ile------	747
HIVELI	---------Asn--	747

HIVHXB2	LysGluIleValAlaSerCysAspLysCysGlnLeuLysGlyGluAlaMetHisGlyGln	768
HIVBH102	--	780
HIVBH5	--	780
HIVPV22	--	780
HIVBRU	--	780
HIVMN	--	677
HIVSF2	--	768
HIVRF	--	767
HIVMAL	--	767
HIVELI	--	767

HIVHXB2	ValAspCysSerProGlyIleTrpGlnLeuAspCysThrHisLeuGluGlyLysValIle	788
HIVBH102	--	800
HIVBH5	--	800
HIVPV22	--	800
HIVBRU	--	800
HIVMN	--	697
HIVSF2	--Ile---	788
HIVRF	--Ile---	787
HIVMAL	--Ile---	787
HIVELI	--	787

Table 1 cont'd

HIVHXB2	LeuValAlaValHisValAlaSerGlyTyrIleGluAlaGluValIleProAlaGluThr	808
HIVBH102	--	820
HIVBH5	--	820
HIVPV22	--	820
HIVBRU	--	820
HIVMN	--	820
HIVSF2	--	717
HIVRF	--	808
HIVMAL	Ile---	807
HIVELI	--	807

HIVHXB2	GlyGlnGluThrAlaTyrPheLeuLeuLysLeuAlaGlyArgTrpProValLysThrIle	828
HIVBH102	--	840
HIVBH5	--	840
HIVPV22	--	840
HIVBRU	--	840
HIVMN	--	840
HIVSF2	--	737
HIVRF	--	828
HIVMAL	---------------------Ile------------------------------Val---	827
HIVELI	---------------------Ile------------------------------ValVal	827
	--ValVal	827

HIVHXB2	HisThrAspAsnGlySerAsnPheThrGlyAlaThrValArgAlaAlaCysTrpTrpAla	848
HIVBH102	---------------------------Ser---------Lys------------------	860
HIVBH5	---------------------------Ser---------Lys------------------	860
HIVPV22	---------------------------Ser---------Lys------------------	860
HIVBRU	---------------------------SerThr------Lys------------------	860
HIVMN	---------------Pro---------SerThr------Lys---------------Thr	860
HIVSF2	---------------------------SerThr------Lys------------------	757
HIVRF	---------------------------SerThr------Lys------------------	848
HIVMAL	---------------------------Ser---Ala---Lys------------------	847
HIVELI	---------------------------Ser---Ala---Lys------------------	847
		847

HIVHXB2	GlyIleLysGlnGluPheGlyIleProTyrAsnProGlnSerGlnGlyValValGluSer	868
HIVBH102	--	880
HIVBH5	--	880
HIVPV22	--	880
HIVBRU	--	880
HIVMN	--	880
HIVSF2	---Ile------	777
HIVRF	--	868
HIVMAL	Asn---	867
HIVELI	--	867
	--	867

Table 1 cont'd

HIVHXB2	MetAsnLysGluLeuLysLysIleIleGlyGlnValArgAspGlnAlaGluHisLeuLys	888
HIVBH102	--	900
HIVBH5	--	900
HIVPV22	--	900
HIVBRU	--	900
HIVMN	--	797
HIVSF2	------Asn---	888
HIVRF	---------Gln------Gln---------------------------------------	887
HIVMAL	---------------------------------------Glu------------------	887
HIVELI	--	887

HIVHXB2	ThrAlaValGlnMetAlaValPheIleHisAsnPheLysArgLysGlyGlyIleGlyGly	908
HIVBH102	--	920
HIVBH5	--	920
HIVPV22	--	920
HIVBRU	--	920
HIVMN	Arg---	817
HIVSF2	--	908
HIVRF	--	907
HIVMAL	--	907
HIVELI	--ArgArg------------	907

HIVHXB2	TyrSerAlaGlyGluArgIleValAspIleIleAlaThrAspIleGlnThrLysGluLeu	928
HIVBH102	--	940
HIVBH5	--	940
HIVPV22	--	940
HIVBRU	--	940
HIVMN	------------------------Gly---------------------------------	837
HIVSF2	--	928
HIVRF	--	927
HIVMAL	---------------------Ile---Met------------------------------	927
HIVELI	---------------------Ile------------------------------------	927

HIVHXB2	GlnLysGlnIleThrLysIleGlnAsnPheArgValTyrTyrArgAspSerArgAsnSer	948
HIVBH102	---Pro	960
HIVBH5	---Pro	960
HIVPV22	---Pro	960
HIVBRU	--AspPro	960
HIVMN	--AspPro	857
HIVSF2	--AsnLysAspPro	948
HIVRF	--AspPro	947
HIVMAL	--Asn---AspPro	947
HIVELI	------------Ile--AspPro	947

Table 1 cont'd

HIVHXB2	LeuTrpLysGlyProAlaLysLeuLeuTrpLysGlyGluGlyAlaValValIleGlnAsp	968
HIVBH102	--	980
HIVBH5	--	980
HIVPV22	--	980
HIVBRU	--	980
HIVMN	--	877
HIVSF2	--	968
HIVRF	------------His---	967
HIVMAL	Ile---	967
HIVELI	Ile---	967

sor cds start ->

HIVHXB2	AsnSerAspIleLysValValProArgArgLysAlaLysIleIleArgAspTyrGlyLys	988
HIVBH102	--	1000
HIVBH5	--	1000
HIVPV22	--	1000
HIVBRU	--	1000
HIVMN	---Asn---------------------------------Val------------------	897
HIVSF2	--	988
HIVRF	--	987
HIVMAL	--	987
HIVELI	Lys------------------------------Val------------------------	987

HIVHXB2	GlnMetAlaGlyAspAspCysValAlaSerArgGlnAspGluAsp+++	1004
HIVBH102	--	1016
HIVBH5	--	1016
HIVPV22	--	1016
HIVBRU	--	1016
HIVMN	---Thr--	913
HIVSF2	--	1004
HIVRF	--	1003
HIVMAL	---------------------------GlyGly---------------	1003
HIVELI	--	1003

TABLE 2

HIV-1 pol gene HIVHXB2 Sequence
Data from Human Retroviruses and AIDS, 1988
Los Alamos National Laboratory

```
     AcNPV-HIVWHpol Virus
                                          RBS
                                   Bam HI       ti
                                5'-GGATCCTATAAATATG  tttttta gggaagatct
          pol cds start (NH2-terminus uncertain) ->

2101 ggccttccta caagggaagg ccagggaatt ttcttcagag cagaccagag ccaacagccc

2161 caccagaaga gagcttcagg tctggggtag agacaacaac tccccctcag aagcaggagc

2221 cgatagacaa ggaactgtat cctttaactt ccctcaggtc actctttggc aacgacccct

2281 cgtcacaaTA Aagatagggg ggcaactaaa ggaagctcta ttagatacag gagcagatga
           <- gag cds end

2341 tacagtatta gaagaaatga gtttgccagg aagatggaaa ccaaaaatga tagggggaat

2401 tggaggtttt atcaaagtaa gacagtatga tcagatactc atagaaatct gtggacataa

2461 agctataggt acagtattag taggacctac acctgtcaac ataattggaa gaaatctgtt

2521 gactcagatt ggttgcactt taaattttcc cattagccct attgagactg taccagtaaa

2581 attaaagcca ggaatggatg gcccaaaagt taaacaatgg ccattgacag aagaaaaaat

2641 aaaagcatta gtagaaattt gtacagagat ggaaaaggaa gggaaaattt caaaaattgg

2701 gcctgaaaat ccatacaata ctccagtatt tgccataaag aaaaaagaca gtactaaatg

2761 gagaaaatta gtagatttca gagaacttaa taagagaact caagacttct gggaagttca

2821 attaggaata ccacatcccg cagggttaaa aaagaaaaaa tcagtaacag tactggatgt

2881 gggtgatgca tatttttcag ttcccttaga tgaagacttc aggaagtata ctgcatttac

2941 catacctagt ataaacaatg agacaccagg gattagatat cagtacaatg tgcttccaca

3001 gggatggaaa ggatcaccag caatattcca aagtagcatg acaaaaatct tagagccttt

3061 tagaaaacaa aatccagaca tagttatcta tcaatacatg gatgatttgt atgtaggatc

3121 tgacttagaa atagggcagc atagaacaaa aatagaggag ctgagacaac atctgttgag

3181 gtggggactt accacaccag acaaaaaaca tcagaaagaa cctccattcc tttggatggg

3241 ttatgaactc catcctgata aatggacagt acagcctata gtgctgccag aaaaagacag

3301 ctggactgtc aatgacatac agaagttagt ggggaaattg aattgggcaa gtcagattta

3361 cccagggatt aaagtaaggc aattatgtaa actccttaga ggaaccaaag cactaacaga

3421 agtaatacca ctaacagaag aagcagagct agaactggca gaaaacagag agattctaaa
```

Table 2 cont'd

```
3481 agaaccagta catggagtgt attatgaccc atcaaaagac ttaatagcag aaatacagaa

3541 gcaggggcaa ggccaatgga catatcaaat ttatcaagag ccatttaaaa atctgaaaac

3601 aggaaaatat gcaagaatga ggggtgccca cactaatgat gtaaaacaat taacagaggc

3661 agtgcaaaaa ataaccacag aaagcatagt aatatgggga aagactccta aatttaaact

3721 gcccatacaa aaggaaacat gggaaacatg gtggacagag tattggcaag ccacctggat

3781 tcctgagtgg gagtttgtta ataccccctcc cttagtgaaa ttatggtacc agttagagaa

3841 agaacccata gtaggagcag aaaccttcta tgtagatggg gcagctaaca gggagactaa

3901 attaggaaaa gcaggatatg ttactaatag aggaagacaa aaagttgtca ccctaactga

3961 cacaacaaat cagaagactg agttacaagc aatttatcta gctttgcagg attcgggatt

4021 agaagtaaac atagtaacag actcacaata tgcattagga atcattcaag cacaaccaga

4081 tcaaagtgaa tcagagttag tcaatcaaat aatagagcag ttaataaaaa aggaaaaggt

4141 ctatctggca tgggtaccag cacacaaagg aattggagga aatgaacaag tagataaatt

4201 agtcagtgct ggaatcagga aagtactatt tttagatgga atagataagg cccaagatga

4261 acatgagaaa tatcacagta attggagagc aatggctagt gattttaacc tgccacctgt

4321 agtagcaaaa gaaatagtag ccagctgtga taaatgtcag ctaaaaggag aagccatgca

4381 tggacaagta gactgtagtc caggaatatg gcaactagat tgtacacatt tagaaggaaa

4441 agttatcctg gtagcagttc atgtagccag tggatatata gaagcagaag ttattccagc

4501 agaaacaggg caggaaacag catattttct tttaaaatta gcaggaagat ggccagtaaa

4561 aacaatacat actgacaatg gcagcaattt caccggtgct acggttaggg ccgcctgttg

4621 gtgggcggga atcaagcagg aatttggaat tccctacaat ccccaaagtc aaggagtagt

4681 agaatctatg aataaagaat taaagaaaat tataggacag gtaagagatc aggctgaaca

4741 tcttaagaca gcagtacaaa tggcagtatt catccacaat tttaaaagaa aaggggggat

4801 tggggggtac agtgcagggg aaagaatagt agacataata gcaacagaca tacaaactaa

4861 agaattacaa aaacaaatta caaaaattca aaattttcgg gtttattacA Gggacagcag
                                                            /\ 3'sj.

4921 aaattcactt tggaaaggac cagcaaagct cctctggaaa gGTgaagggg cagtagtaat
                                            5'sj /\

4981 acaagataat agtgacataa aagtagtgcc aagaagaaaa gcaaagatca ttagggattA
                                                 sor 23 kD cds start ->
                                                                Bam HI
5041 TGgaaaacag atggcaggtg atgattgtgt ggcaagtaga caggatgagg atTAGGATCC-3'
                                                 Crossover      <- pol end
                                                 linker sequence
```

TABLE 3

HIV-1 pol gene
HIVHXB2 Sequence
Data from Human Retroviruses and AIDS, 1988
Los Alamos National Laboratory

```
AcNPV-HIVWHpol Virus
                                               RBS
                                         Bam HI         ti(AcNPV-HIVWHpol start)
                                       5'-GGATCCTATAAATATGtttttta gggaagatct
                    pol cds start (NH2-terminus uncertain) ->

2101 ggccttccta caagggaagg ccagggaatt ttcttcagag cagaccagag ccaacagccc

2161 caccagaaga gagcttcagg tctggggtag agacaacaac tccccctcag aagcaggagc

2221 cgatagacaa ggaactgtat cctttaactt ccctcaggtc actctttggc aacgacccct

2281 cgtcacaaTA Aagatagggg ggcaactaaa ggaagctcta ttagatacag gagcagatga

AcNPV-HIVYKpol      Bam HI   RBS  ti
Virus            5'-GGATCCTATAAATATG (ti;AcNPV-HIVYKpol start)

2341 tacagtatta gaagaaatga gtttgccagg aagatggaaa ccaaaaatga tagggggaat

2401 tggaggtttt atcaaagtaa gacagtatga tcagatactc atagaaatct gtggacataa

2461 agctataggt acagtattag taggacctac acctgtcaac ataattggaa gaaatctgtt

2521 gactcagatt ggttgcactt taaattttcc cattagccct attgagactg taccagtaaa

2581 attaaagcca ggaatggatg gcccaaaagt taaacaatgg ccattgacag aagaaaaaat

2641 aaaagcatta gtagaaattt gtacagagat ggaaaaggaa gggaaaattt caaaaattgg

2701 gcctgaaaat ccatacaata ctccagtatt tgccataaag aaaaaagaca gtactaaatg

2761 gagaaaatta gtagatttca gagaacttaa taagagaact caagacttct gggaagttca

2821 attaggaata ccacatcccg cagggttaaa aaagaaaaaa tcagtaacag tactggatgt

2881 gggtgatgca tatttttcag ttcccttaga tgaagacttc aggaagtata ctgcatttac

2941 catacctagt ataaacaatg agacaccagg gattagatat cagtacaatg tgcttccaca

3001 gggatggaaa ggatcaccag caatattcca aagtagcatg acaaaaatct tagagccttt

3061 tagaaaacaa aatccagaca tagttatcta tcaatacatg gatgatttgt atgtaggatc

3121 tgacttagaa atagggcagc atagaacaaa aatagaggag ctgagacaac atctgttgag

3181 gtggggactt accacaccag acaaaaaaca tcagaaagaa cctccattcc tttggatggg

3241 ttatgaactc catcctgata aatggacagt acagcctata gtgctgccag aaaaagacag

3301 ctggactgtc aatgacatac agaagttagt ggggaaattg aattgggcaa gtcagattta

3361 cccagggatt aaagtaaggc aattatgtaa actccttaga ggaaccaaag cactaacaga

3421 agtaatacca ctaacagaag aagcagagct agaactggca gaaaacagag agattctaaa
```

Table 3 cont'd

```
3481 agaaccagta catggagtgt attatgaccc atcaaaagac ttaatagcag aaatacagaa
3541 gcaggggcaa ggccaatgga catatcaaat ttatcaagag ccatttaaaa atctgaaaac
3601 aggaaaatat gcaagaatga ggggtgccca cactaatgat gtaaaacaat taacagaggc
3661 agtgcaaaaa ataaccacag aaagcatagt aatatgggga aagactccta aatttaaact
3721 gcccatacaa aaggaaacat gggaaacatg gtggacagag tattggcaag ccacctggat
3781 tcctgagtgg gagtttgtta atacccctcc cttagtgaaa ttatggtacc agttagagaa
3841 agaacccata gtaggagcag aaaccttcta tgtagatggg gcagctaaca gggagactaa
3901 attaggaaaa gcaggatatg ttactaatag aggaagacaa aaagttgtca ccctaactga
3961 cacaacaaat cagaagactg agttacaagc aatttatcta gctttgcagg attcgggatt
4021 agaagtaaac atagtaacag actcacaata tgcattagga atcattcaag cacaaccaga
4081 tcaaagtgaa tcagagttag tcaatcaaat aatagagcag ttaataaaaa aggaaaaggt
4141 ctatctggca tgggtaccag cacacaaagg aattggagga aatgaacaag tagataaatt
4201 agtcagtgct ggaatcagga aagtactatt tttagatgga atagataagg cccaagatga
4261 acatgagaaa tatcacagta attggagagc aatggctagt gattttaacc tgccacctgt
4321 agtagcaaaa gaaatagtag ccagctgtga taaatgtcag ctaaaaggag aagccatgca
4381 tggacaagta gactgtagtc caggaatatg gcaactagat tgtacacatt tagaaggaaa
4441 agttatcctg gtagcagttc atgtagccag tggatatata gaagcagaag ttattccagc
4501 agaaacaggg caggaaacag catatttttct tttaaaatta gcaggaagat ggccagtaaa
4561 aacaatacat actgacaatg gcagcaattt caccggtgct acggttaggg ccgcctgttg
4621 gtgggcggga atcaagcagg aatttggaat tccctacaat ccccaaagtc aaggagtagt
4681 agaatctatg aataaagaat taaagaaaat tataggacag gtaagagatc aggctgaaca
4741 tcttaagaca gcagtacaaa tggcagtatt catccacaat tttaaaagaa aaggggggat
4801 tggggggtac agtgcagggg aaagaatagt agacataata gcaacagaca tacaaactac
4861 agaattacaa aaacaaatta caaaaattca aaattttcgg gtttattacA Gggacagcag
                                                            /\ 3'sj
4921 aaattcactt tggaaaggac cagcaaagct cctctggaaa gGTgaagggg cagtagtaat
                                            5'sj /\
```

Table 3 cont'd

```
4981 acaagataat agtgacataa aagtagtgcc aagaagaaaa gcaaagatca ttagggattA
                                                          Bam HI
5041 TGgaaaacag atggcaggtg atgattgtgt ggcaagtaga caggatgagg atTAGGATCC
                                                              Sph I
                                                              GCATG-3'
                                                        <- pol end

5101 ggaaaagttt agtaaaacac catatgtatg tttcagggaa agctagggga tggttttata

5161 gacatcacta tgaaagccct catccaagaa taagttcaga agtacacatc ccactagggg

5221 atgctagatt ggtaataaca acatattggg gtctgcatac aggagaaaga gactggcatt

5281 tgggtcaggg agtctccata gaatggagga aaaagagata tagcacacaa gtagaccctg

5341 aactagcaga ccaactaatt catctgtatt actttgactg tttttcAGac tctgctataa
                                                   /\ 3'sj

5401 gaaaggcctt attaggacac atagttagcc ctaggtgtga atatcaagca ggacataaca

5461 agGTaggatc tctacaatac ttggcactag cagcattaat aacaccaaaa aagataaagc
 5'sj /\

5521 caccttttgcc tagtgttacg aaactgacag aggatagATG gaacaagccc cagaagacca
                          R orf cds start ->

5581 agggccacag agggagccac acaatgaatg gacacTAGag cttttagagg agcttaagaa
                                        <- sor 23 kD cds end

5641 tgaagctgtt agacattttc ctaggatttg gctccatggc ttagggcaac atatctatga

5701 aacttatggg gatacttggg caggagtgga agccataata agaattctgc aacaactgct

5761 gtttatccat tttcAGaatt gggtgtcgac aTAGcagaat aggcgttact cgacagagga
                   /\ 3'sj              <- R orf cds end

5821 gagcaagaaA TGgagccagt agatcctaga ctagagccct ggaagcatcc aggaagtcag
tat cds start ->

5881 cctaaaactg cttgtaccaa ttgctattgt aaaaagtgtt gctttcattg ccaagtttgt

5941 ttcataacaa aagccttagg catctcctAT GgcAGgaaga agcggagaca gcgacgaaga
               trs/art cds start ->      /\ 3'sj

6001 gctcatcaga acagtcagac tcatcaagct tctctatcaa agcaGTaagt agtacatgta
                       (tat, trs/art, 27 kD) 5'sj /\

6061 AcGcaaccta taccaatagt agcaatagta gcattagtag tagcaataat aatagcaata
U orf ->

6121 gttgtgtggt ccatagtaat catagaatat aggaaaatat taagacaaag aaaaatagac
```

CLAIMS:

1. The use of a polypeptide as a reagent in a diagnostic test for HIV infection or as a vaccine against HIV infection characterized in that said polypeptide comprises a substantial portion of each of more than one of the enzymes coded for by the HIV-pol gene.

2. The use claimed in Claim 1 characterized in that said polypeptide comprises a plurality of enzymes selected from HIV-pol protease, HIV-pol reverse transcriptase, HIV-pol RNAse H and HIV-pol Integrase.

3. The use claimed in Claim 2 characterized in that said polypeptide comprises substantial portions of all four of said enzymes.

4. The use claimed in Claim 2 characterized in that said polypeptide omits at least that part of the amino acid sequence of the HIV-pol protease gene which codes for the active site responsible for proteolytic activity.

5. The use claimed in Claim 3 characterized in that said polypeptide omits at least that part of the amino acid sequence of the HIV-pol protease gene which codes for the active site responsible for proteolytic activity.

6. A diagnostic kit for detecting antibodies to HIV antigens characterized in that said kit contains as a test reagent, a polypeptide as defined in Claim 1, Claim 2, Claim 3, Claim 4 or Claim 5.

7. A vaccine for protecting an individual against HIV infection comprising a polypeptide and a pharmaceutically acceptable carrier, characterized in that said polypeptide is as claimed in Claim 1, Claim 2, Claim 3, Claim 4 or Claim 5.

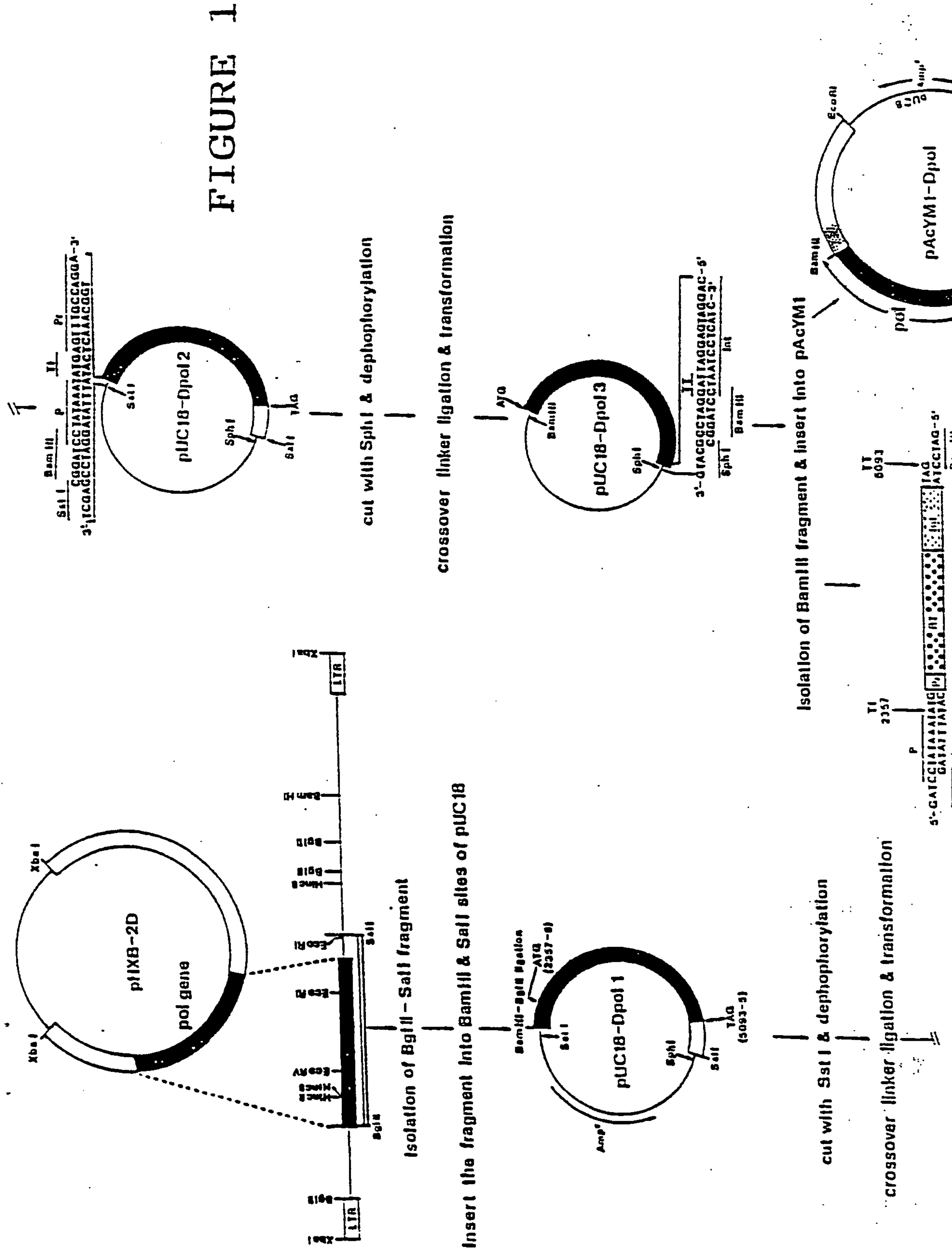
8. A polypeptide comprising a substantial portion of each of more than one of the enzymes coded for by the HIV-pol gene characterised by omitting at least that part of the amino acid sequence of the HIV-pol protease gene which codes for the active site responsible for proteolytic activity.

9. A polypeptide as claimed in Claim 8 characterized by comprising sequences of a plurality of enzymes selected from HIV-pol protease, HIV-pol reverse transcriptase, HIV-pol RNase H and HIV-pol Integrase.

10. A polypeptide according to Claim 9 characterized in that said polypeptide contains substantial portions of all four of said enzymes.

11. A polypeptide according to Claim 8 characterized in that said polypeptide has an amino acid sequence substantially as shown in Table 3 beginning with the amino acid Met marked "AcNPV-HIVYKpol starts".

FIGURE 1


pIIXB-2D
pol gene
XbaI
XbaI
LTR
BglII
BglII
EcoRV
HincII
EcoRI
SalI
BglII
BglII
HincII
BamHI
LTR
XbaI
Isolation of BglII - SalI fragment
Insert the fragment into BamHI & SalI sites of pUC18
pUC18-Dpol1
BamHI-BglII fusion
ATG (2357-9)
SalI
SphI
SalI
TAG (5093-5)
Amp^r
cut with SstI & dephophorylation
crossover linker ligation & transformation
SstI
BamHI
P
II
Pt
3'-TCGAGCCTAGGATATTTTATACTCAAACGGT
CGGATCCTATAAAATATGAGTTTGCCAGGA-3'
pUC18-Dpol2
SalI
SphI
SalI
TAG
cut with SphI & dephophorylation
crossover linker ligation & transformation
pUC18-Dpol3
ATG
BamHI
SphI
3'-GTACGGCTAGGATTAGGAGTAGGAC-5'
CGGATCCTAATCCTCATC-3'
SphI
BamHI
II
Int
Isolation of BamHI fragment & insert into pAcYM1
5'-GATCCTATAAAATATG
GATATTTTATAC
BamHI
P
TI 2357
Pr
RT
Int
TT 5093
TAG
ATCCTAG-5'
BamHI
pAcYM1-Dpol
BamHI
BamHI
pol
EcoRI
EcoRI
Amp^r
ori
pUC8

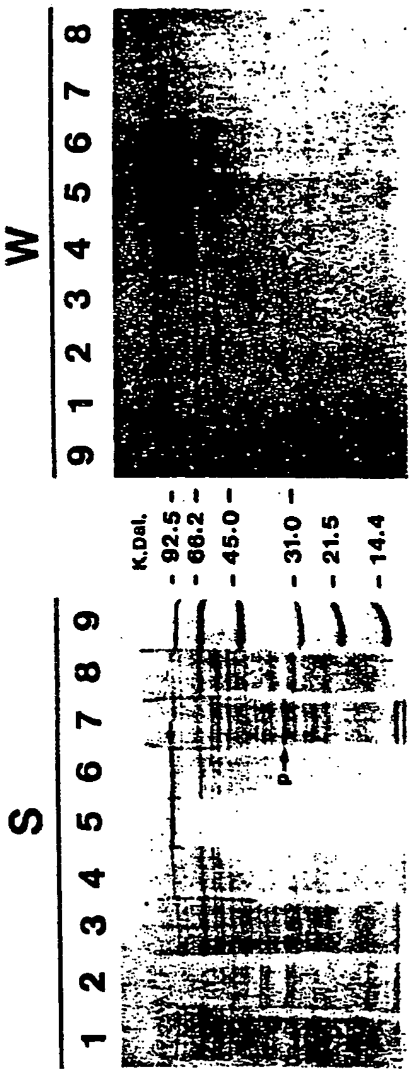
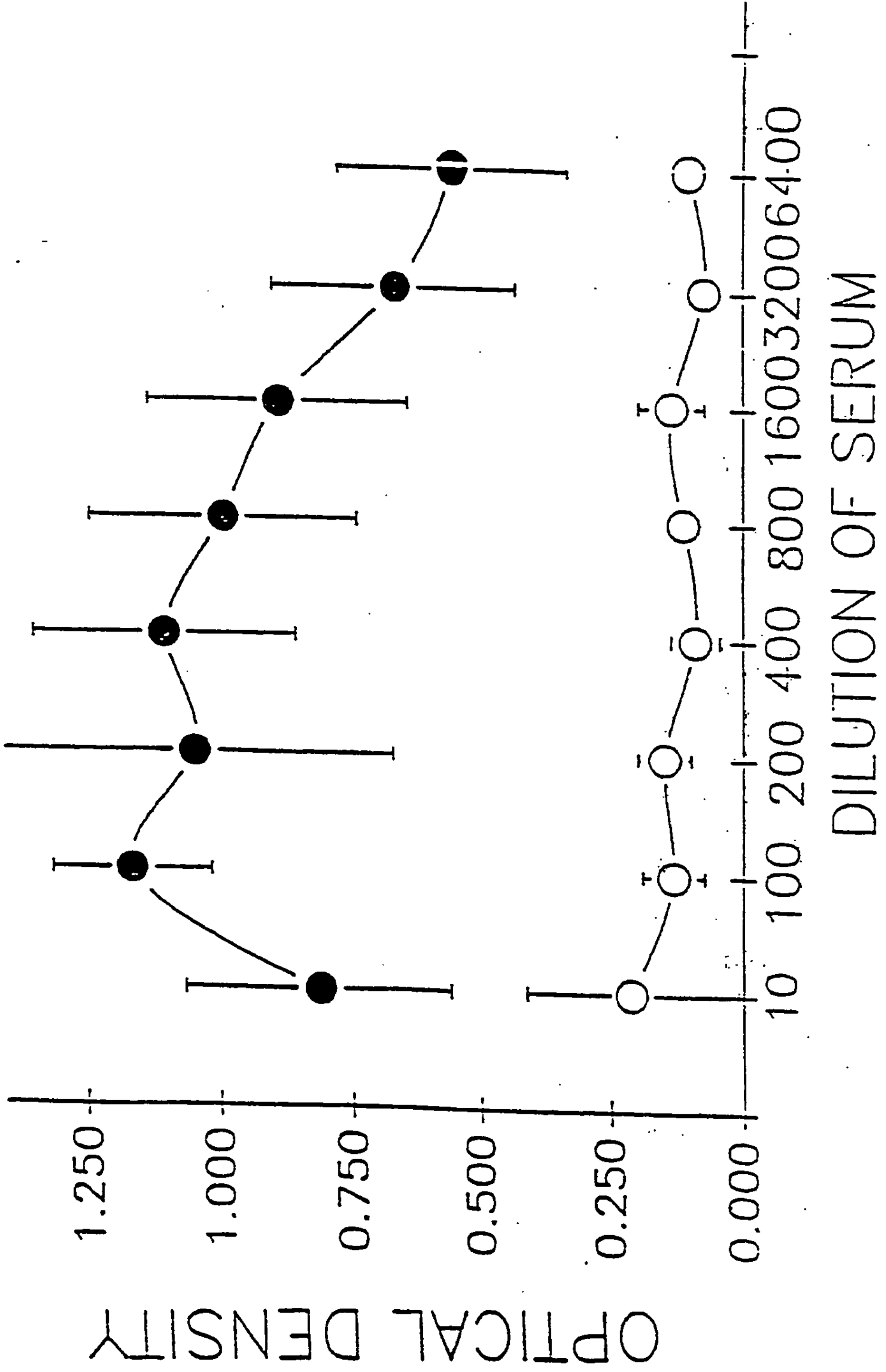


FIG. 2

FIGURE 3

SERUM FROM HIV—1+ PATIENTS (N=5)



INTERNATIONAL SEARCH REPORT

International Application No PCT/CA 90/00062

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC^5: G 01 N 33/569, A 61 K 39/21, C 07 K 15/04, C 12 N 9/12, C 12 N 9/22, C 12 N 9/16, C 12 N 9/50, C 12 N 9/49

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC^5	C 12 N, A 61 K, G 01 N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	WO, A, 87/04728 (CAMBRIDGE BIOSCIENCE CORPORATION) 13 August 1987 see pages 31-33 --	1-8
Y	WO, A, 87/07296 (THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK) 3 December 1987 see pages 5-7 --	1-8
P,X	EP, A, 0322922 (MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER WISSENSCHAFTEN e.V) 5 July 1989 see the whole document -- ./.	1-8

* Special categories of cited documents: [10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search 19th June 1990	Date of Mailing of this International Search Report 18. 07. 90
International Searching Authority EUROPEAN PATENT OFFICE	Signature of Authorized Officer H. Daniels H. DANIELS

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	Science, vol. 236, 17 April 1987, W.G. Farmerie et al.: "Expression and processing of the AIDS virus reverse transcriptase in Escherichia coli", pages 305-308, see figure 1 --	1-8
A	EP, A, 0196056 (CHIRON CORPORATION) 1 October 1986 see example II --------------------------	1-8

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

CA 9000062
SA 35054

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A- 8704728	13-08-87	US-A- 4753873	28-06-88
		US-A- 4734362	29-03-88
		AU-A- 7022787	25-08-87
		AU-A- 7081987	25-08-87
		EP-A- 0233044	19-08-87
		EP-A- 0233045	19-08-87
		JP-T- 63502957	02-11-88
		JP-T- 63502958	02-11-88
		OA-A- 8687	31-03-89
		OA-A- 8762	31-03-89
		WO-A- 8704726	13-08-87
WO-A- 8707296	03-12-87	AU-A- 7512287	22-12-87
		JP-T- 63503356	08-12-88
EP-A- 0322922	05-07-89	None	
EP-A- 0196056	01-10-86	CA-A- 1260858	26-09-89
		JP-A- 61268193	27-11-86
		US-A- 4751180	14-06-88

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